DNA Identification of Commercial Ginseng Samples

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An investigation was performed with the objective of developing a DNA-based protocol for the identification of commercial samples of the herbal compound ginseng. There are currently two major herbal products referred to as ginseng. They are Korean or Chinese ginseng (*Panax ginseng*) and American ginseng (*Panax quinquefolius*). The market for ginseng in the United States is estimated to be ∼$300 million annually. Current tests for ginseng species identification rely on expert botanical identification of fresh plant/root specimens or on biochemical characterization of active and marker compounds (e.g., ginsenosides). For the determination of the feasibility of ginseng identification by DNA analysis, a strategy based on the direct DNA sequence analysis of the nuclear ribosomal internal transcribed spacer region was developed. Other genetic tests included sequence analysis of the chloroplast ribulose 1,5-bisphosphate carboxylase large subunit gene and DNA fingerprinting by the rapid amplification of polymorphic DNA technique. To confirm the results, each ginseng sample was identified using high-performance liquid chromatography. All methods were successful in distinguishing American from Korean ginseng. In addition, the protocol was improved for the isolation of genomic and plastid DNA from commercial ginseng preparations by incorporating an impact homogenization step into the standard column chromatography purification procedure.

Keywords: *DNA testing; ginseng; Panax; herbal compounds; alternative medicine*

INTRODUCTION

The herbal supplement market has been growing exponentially in the past few years. A number of herbal preparations or medicinal botanicals have recently topped the $100 million annual revenue milestone in the United States market (Zeisel, 1999). Top sellers include ginkgo, St. John's wort, ginseng, echinacea, and saw palmetto. As with any natural product, supply depends on adequate production from growers and harvesters. Little or no regulation is currently in place to oversee the quality and identity of raw herbal products. As the monetary incentive for herbal production increases, it becomes critical that robust identification procedures are available. As an example, ginseng in its powdered form (the most common type of ginseng preparation) is not identifiable by smell, taste, or appearance (Evans, 1996). Products sold as American or Korean ginseng could be substituted for each other or with different herbal compounds without the consumer's knowledge. For that matter, any adulterant or substitute would be difficult to detect without expert analysis.

The ginseng root has been used in traditional medicine for >2000 years (Evans, 1996). A wide range of therapeutic uses has been reported, including general disorders such as aging and cancer. Currently, the primary use of ginseng is for its "adaptogenic" or stress-protective effect (Li and Harries, 1996). Specific pharmocologic effects have been difficult to establish, although American ginseng has been documented to help regulate blood sugar levels after a high-sugar meal (Fontanarosa and Lundberg, 1998; Vuksan et al., 2000). Ginseng is often sold as either "red" or "white" ginseng, which refers to the processing of the root material (Cutler and Cutler, 2000). Raw or nonprocessed ginseng roots, normally tan to white in color, are sometimes treated with steam at 100 °C for a period of time to preserve them from microbial contamination. The result of these high temperatures is reddening of the root material (red ginseng).

Current herbal identification methods rely on microscopic characteristics, thin-layer chromatography (TLC) and high-performance liquid chromatography (HPLC) (Evans, 1996). These techniques can be subject to misinterpretation (microscopic analysis), or molecular standards (TLC and HPLC) can be difficult to obtain. Because herbal compounds come from botanical specimens and often contain intact cellular material after commercial processing, it should be possible to isolate DNA from samples and authenticate them by genetic analysis. The biotechnology revolution has given scientists an arsenal of molecular tools that allow genetic analysis of almost any type of biological specimen, including animal, agricultural, and herbal products (Moritz and Hillis, 1996; Wolf et al., 1999). Specifically, Polymerase Chain Reaction (PCR) allows the amplification of small amounts of DNA from biological samples, even highly degraded samples, which can then be used for further molecular analysis (Thomas et al., 1994; Chicca et al., 2000). PCR has greatly increased our ability to identify life forms, even some that have been dead for many thousands of years (Audic and Beraud-Colomb, 1997).

In this paper, we describe the analysis of commercial ginseng samples, both Amercian (*Panax quinquefolius*) and Korean ginseng (*Panax ginseng*), by both molecular

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Table 1. Ginseng Sample Sources, ITS Sequence Results, and Sample Identities

sample	source	label; type	118	237	411	425	identity
			sequence results[a]	
1	M	American; fresh root	C	G	T	C	American
2	A	American; dried root	C	G	T	C	American
3	A	American; dried root	C	G	T	C	American
4	B	American; powder	C	G	T	C	American
5	C	American; powder	C	G	T	C	American
6	D	American; powder	C	G	T	C	American
7	E	American; powder	C	G	T	C	American
8	F	American; capsule	C	G	T	C	American
9	G	American; capsule	C	G	T	C	American
10	H	American; capsule	C	G	T	C	American
11	I	Korean; powder	A	A	C	T	Korean
12	N	Red Korean; powder	A	A	C	T	Korean
13	D	Korean; powder	A	A	C	T	Korean
14	E	Korean; powder	A	A	C	T	Korean
15	E	Chinese; powder	A	A	C	T	Korean
16	J	Korean; capsule	–	–	–	–	soybean
17	F	Korean; capsule	A	A	C	T	Korean
18	H	Korean; capsule	A	A	C	T	Korean
19	H	Red Korean; capsule	A	A	C	T	Korean
20	K	Korean; tablet	–	–	–	–	Soybean
G1	D	American; powder	C	G	T	C	American
G2	E	American; powder	C	G	T	C	American
G3	L	American; dried root	C	G	T	C	American
G4	D	Korean; powder	A	A	C	T	Korean
G5	E	Korean; powder	A	A	C	T	Korean
G6	L	Korean; dried root	A	A	C	T	Korean

[a] Numbers refer to nucleotide positions of the 18S–28S ribosomal ITS region. The dashes represent DNA sequence that does not match the ginseng ITS region.

genetic and reverse-phase HPLC techniques. Sample preparations included whole root, powdered root, capsules, and tablets. Our results show that all types of commercial preparations of ginseng can be authenticated by direct DNA sequence analysis of the genomic ribosomal internal transcribed spacer (ITS) region (Wen and Zimmer, 1996). We have also identified a set of oligonucleotide primers used in random amplified polymorphic DNA (RAPD) analysis that are polymorphic for Korean and American ginsengs.

MATERIALS AND METHODS

Ginseng Samples. Samples of commercial ginseng, in the form of whole root, powder, capsule, or tablet, were purchased from local and national herbal health care stores or were present in the botanical collection at The University of the Sciences in Philadelphia. To protect the manufacturers' identities, the sample sources are labeled A–N (Table 1). Two additional samples were used as dried whole root controls. These samples (G3 and G6) were purchased in Qingdao, People's Republic of China, during a trip by one of the authors.

DNA Purification. Ginseng plant material (~300 mg) in the form of either whole root or tablet was first minced with a sterile scalpel and pulverized to a powder using a sterilized mortar and pestle. Ginseng samples in powder and capsule form were used directly in the DNA isolation procedure. A DNA isolation kit (DNeasy, No. 69103) (Qiagen Inc., Valencia, CA) was used as described in the manufacturer's instructions with slight modifications (note that Qiagen buffers are proprietary, and recipes are not normally available). Three hundred milligrams of the prepared or powdered ginseng sample was used in the purification procedure. Before sample elution, the columns were dried at 37 °C for 5 min to evaporate residual ethanol. Samples were eluted in a total volume of 200 μL of TE buffer [10 mM Tris-HCl, 1 mM EDTA (pH 8.0)]. Fifteen microliters (15 μL) of each ginseng DNA sample was analyzed by 1% agarose gel electrophoresis with ethidium bromide staining (Figure 1A) (Sambrook et al., 1989).

A second DNA isolation protocol was used to improve the yield of DNA from ginseng samples. This protocol used

beadmill cell disruption to provide greater homogenization of plant cell material (Kuske et al., 1998). Three hundred milligrams (300 mg) of ginseng powder was placed in a sterile 2 mL screw-cap tube. Approximately 1.0 mL of glass beads (65% 1.0 mm and 35% 0.5 mm) was added to the tube. Qiagen lysis buffer AP1 was preheated to 65 °C, and 1.2 mL of the buffer was added to the tube containing the powdered ginseng and glass bead mixture. The ginseng sample was placed in a Biospec 3110 Mini-BeadBeater (BioSpec Products, Inc., Bartlesville, OK) and shaken for 3 min at 5000 rpm. The sample was then placed at −75 °C for 5 min and then shaken for an additional 5 min at 5000 rpm. The tube was briefly centrifuged and 400 μL of the supernatant removed; this ginseng cell lysate sample was then processed using the Qiagen DNeasy column chromatography system as described above (Figure 1B).

PCR Amplification. PCR amplification was performed on all ginseng DNA samples using oligonucleotide primers specific for the nuclear ribosomal ITS region and for the chloroplast ribulose bisphosphtase large subunit gene (rbcL) (Moritz and Hillis, 1996). The nested oligonucleotide primers that were used in sequencing the ITS region were based on GenBank accession entries for *P. ginseng* (U41680) and *P. quinquefolius* (U41687) (Wen and Zimmer, 1996). The sequences of the PCR and sequencing oligonucleotides for the ITS region are plt-08 (5′-AACAAGGTTTCCGTAGGTGA), plt-09 (5′-TATGCTTAAAY-TCAGCGGGT), plt-10 (5′-CGAACACGTTACAATACCG), and plt-11 (5′-ACCACTTGTCGTGACGTCC). Primers used in the analysis of the rbcL locus are plt-06 (5′-CACCACAAACA-GAAACTAAAGCAAGT) and plt-07 (5′-CTTTAGTAAAAGAT-TGGGCCGAG) (Figure 2). Stock oligonucleotides were resuspended in 0.5× Tris EDTA [5 mM Tris-HCl, 0.5 mM EDTA (pH 8.0)] buffer to a final concentration of 100 μM. PCR samples were prepared by adding 1–2 μL (~5 ng or less) of purified genomic/plastid DNA to a reaction cocktail containing 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 0.001% gelatin, 2.5 mM MgCl$_2$, 1 μM each oligonucleotide primer, and 0.5 unit of Taq DNA polymerase, in a total volume of 20 μL. PCR thermocycler conditions for both the ITS and rbcL loci were as follows: 95 °C, 1 min; 50 °C, 1 min; 72 °C, 2 min, for 35 cycles. PCR samples were analyzed on a 1.3% agarose gel stained with ethidium bromide.

DNA Sequencing and Analysis. PCR products were purified by spin column chromatography (Qiagen Inc.) as described in the manufacturer's instructions. Samples were eluted in a total volume of 30 μL of TE buffer. One to two microliters of purified PCR product (~50 ng) was added to a DNA sequencing reaction containing: 6 μL of FS Taq dye terminator mix (PE Biosystems, Foster City, CA) and 0.2 μM sequencing primer in a total volume of 20 μL. Sequencing thermocycler conditions were 96 °C for 10 s, 50 °C for 5 s, and 60 °C for 4 min, for 25 cycles. DNA termination products were precipitated by adding the entire reaction volume to 75 μL of 70% ethanol/5 mM MgCl$_2$, incubated at room temperature for 20 min, and spun at maximum speed in a microcentrifuge for 20 min. The supernatant was discarded and the pellet dried at 37 °C for 2–3 min. The sample was then resuspended in 25 μL of template suppression reagent (PE Biosystems) and heated at 95 °C for 3 min. After brief centrifugation, the DNA sequencing samples were analyzed on a PE Biosystems 310 prism capillary fluorescent DNA sequencer (61 cm capillary). Electropherograms were generated for each DNA template, and the resulting sequence data were analyzed using the MACDNASIS software program (Hitachi Genetic Systems, Alameda, CA). DNA sequence alignments were performed using the *P. ginseng* and *P. quinquefolius* sequences present in GenBank. DNA sequences that did not match known *Panax* sequences were analyzed with the National Center for Biotechnology Information (NCBI) BLAST (gapped version 2.0) sequence analysis search algorithm (Altschul et al., 1990).

Random Amplification of Polymorphic DNA (RAPD). Purified genomic DNA from ginseng samples were analyzed by RAPD PCR using 10-mer oligonucleotides kits (kits A and Z, Operon Technologies, Inc., Alameda, CA), each containing 20 different oligonucleotides (Williams et al., 1990). Oligonucleotides were resuspended in 0.5× TE buffer to a final

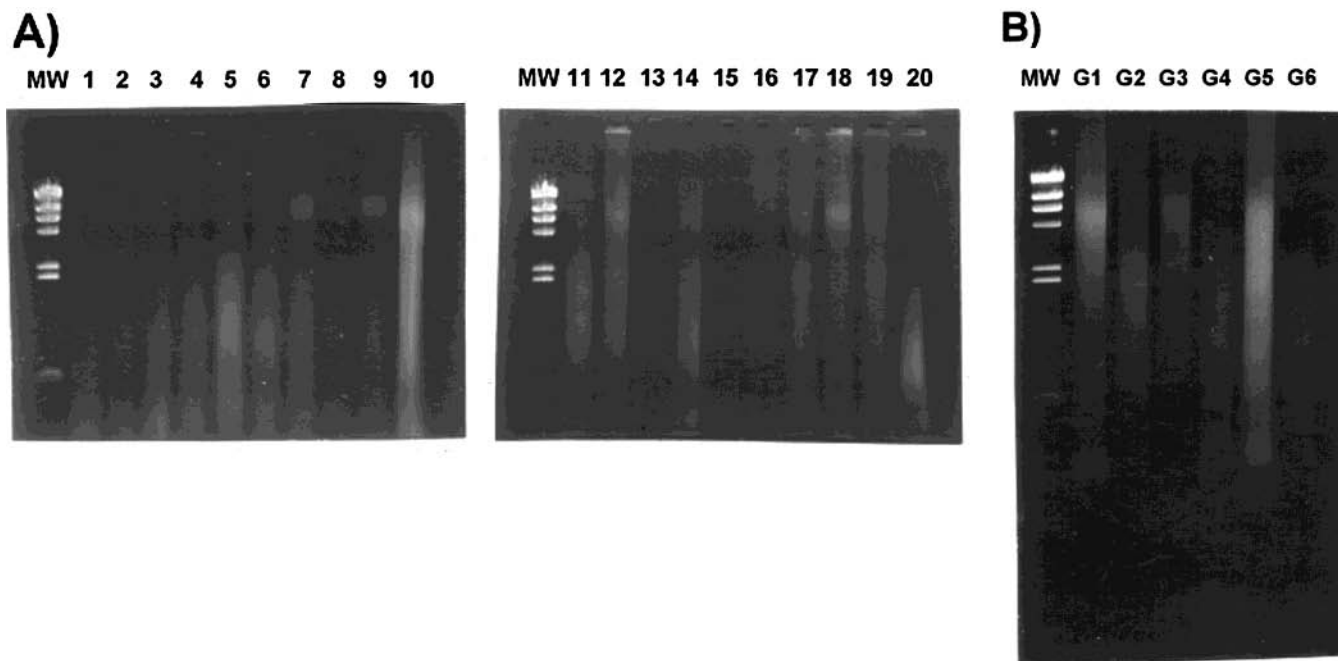**3746** *J. Agric. Food Chem.,* Vol. 48, No. 8, 2000

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Figure 1. Agarose gel electrophoresis of total DNA isolated from commercial ginseng samples: (A) DNA samples isolated by standard plant spin column chromatography; (B) DNA samples isolated using impact homogenization. MW refers to λ/*Hin*dIII molecular weight markers (fragment size from top to bottom: 23130, 9416, 6557, 4361, 2322, 2027, and 564 base pairs). Sample numbers are as described in Table 1.
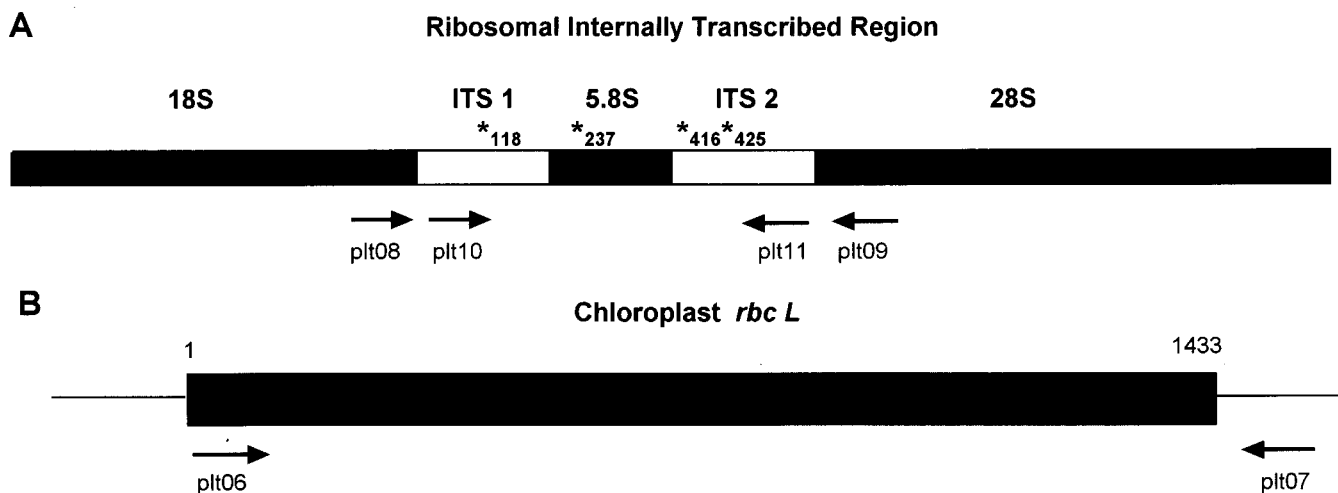

Figure 2. (A) Diagram of the nuclear ribosomal ITS region. Genetic loci are the 18S rRNA small subunit, ITS I, 5.8S rRNA, ITS 2, and 28S rRNA large subunit genes, respectively. Asterisks (*) represent the nucleotide positions in the ginseng ITS region as described in Wen and Zimmer (1996). Plt08, plt10, plt11, and plt09 represent primers used in PCR and DNA sequencing of the ITS locus. (B) Diagram of the chloroplast rbcL gene. Plt06 and plt07 represent the primers used in PCR and DNA sequencing of the rbcL locus.

concentration of 10 μM. All 40 oligonucleotides were used to screen both American and Korean ginseng samples. Subsets of four RAPD oligonucleotides (A2, Z8, Z10, and Z20) were used to demonstrate informative DNA fingerprint patterns. RAPDs were performed by adding 2 μL of genomic DNA ($\sim$50 ng) to a PCR mix containing 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 0.001% gelatin, 2.5 mM MgCl$_2$, $\sim$1.0 μM 10-mer oligonucleotide, and 0.5 unit of Taq DNA polymerase, in a total volume of 20 μL. Thermocycler conditions were as follows: 94 °C for 1 min, 39 °C for 1 min, 72 °C for 2 min for 35 cycles. PCR RAPD results were analyzed on a 1.3% agarose gel and stained with ethidium bromide.

HPLC of Ginseng Extracts. Two grams of each ginseng sample was extracted for 4 h with 200 mL of 90% ethanol using the Soxhlet extraction method (Shugar et al., 1981). This gave a final concentration of $\sim$10 mg of ginseng per 1 mL of solvent. Reverse-phase HPLC was performed using a Beckman Ultrasphere column (4.6 × 250-mm, 5 μm ODS packing) (Beckman

Coulter, Inc., Fullerton, CA). Mobile phase conditions consisted of a gradient elution of acetonitrile (solvent B) and water (solvent A): 0−20 min, 20% B, 20−60 min, 20−42% B; 60−67.5 min, 42−75% B; 67.5−73 min, 75−20% B; 73−75 min, 20% B. Flow rate was 1.3 mL/min. Detection was by UV absorbance spectrophotometry at 203 nm. Ginsenoside HPLC standards (Indofine Chemical Co., Somerville, NJ) were used to verify the identity of chromatographic peaks.

RESULTS

Extraction of DNA from Ginseng Root Preparations. Twenty different ginseng samples were used in the initial DNA purification experiments. Table 1 lists the samples that were used in this study. Samples 1−10 were labeled "American ginseng" (*Panax quinquefolius*), whereas samples 11−20 were labeled "Korean ginseng"
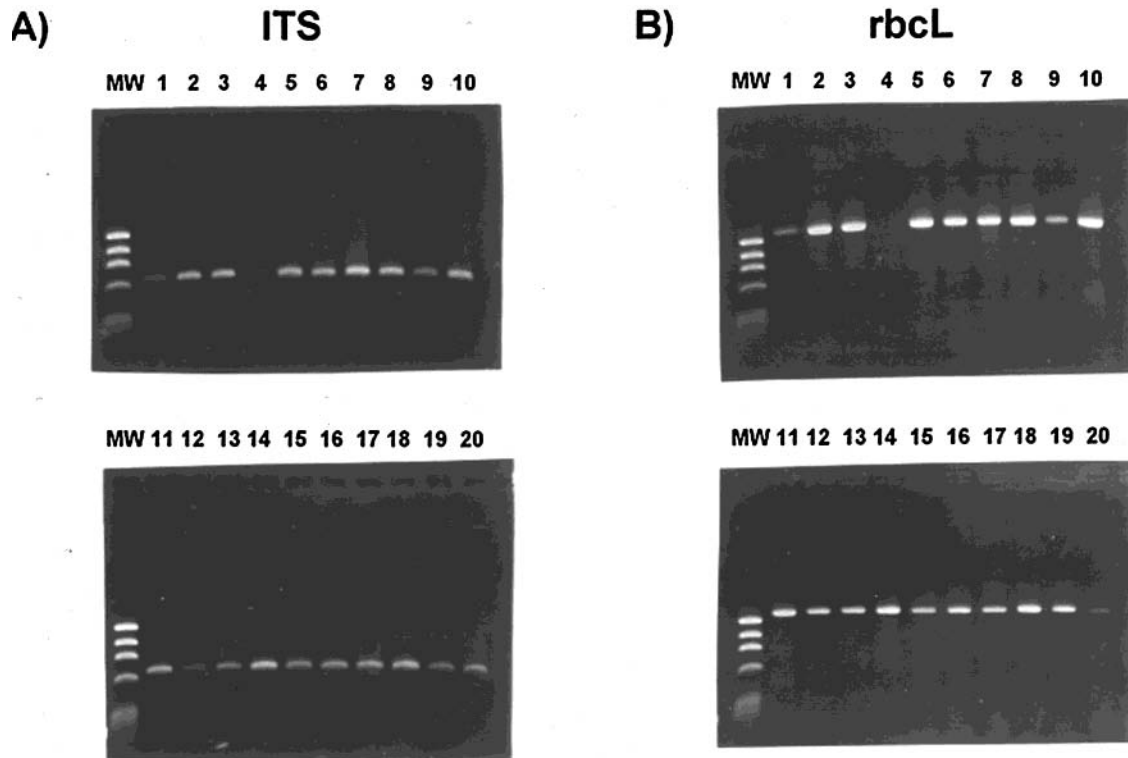
Figure 3. (A) Agarose gel electrophoresis of ITS PCR results from commercial ginseng samples. (B) Agarose gel electrophoresis of rbcL PCR results of commercial ginseng samples. MW refers to *PhiX*174/*Hae*III molecular weight markers [fragment size from top to bottom: 1353, 1078, 872, 603, and (310/281/271) base pairs]. Sample numbers are as in Table 1.

(*Panax ginseng*) by the commercial sources, with the exception that sample 15 was labeled "Chinese ginseng". The sample sources are referred to as A—N to protect the manufacturers's identities. Purified genomic and associated mitochondrial/plastid DNA was analyzed by agarose gel electrophoresis and ethidium bromide staining. Figure 1A shows a representative qualitative and quantitative analysis of the DNA preparations. These initial DNA purification results show that moderately sheared genomic DNA can be isolated from fresh and dried root and ground commercial powder (free and capsule) preparations of ginseng in concentrations (nanogram level) detectable by ethidium bromide stained agarose gel electrophoresis. The most degraded sample of the 20 tested was Korean sample 20, a tablet preparation.

Subsequent PCR analysis of the 20 ginseng DNA samples showed significant variability with respect to the quality and quantity of product. This was especially true concerning RAPD PCR analysis (see below). We employed a second method for ginseng DNA purification using glass bead impact homogenization to improve cell lysis and DNA yield (Kuske et al., 1998). Figure 1B shows an agarose gel electrophoresis analysis of six ginseng samples prepared by this method. Samples G1 and G2 (American) and G4 and G5 (Korean) are the same source samples as 6 and 7 and 13 and 14, respectively. Results show that both the quality and the quantity of the genomic DNA are moderately improved over those of the initial DNA purification procedure. As discussed below, PCR amplification of the DNA from ginseng samples purified by the impact homogenization method was more effective and allowed for higher quality PCR RAPD results.

PCR Amplification of Ginseng Samples. Each of the ginseng DNA samples was subjected to PCR amplification in separate reactions using primers specific

for the genomic ribosomal RNA ITS and the chloroplast ribulose 1,5-bisphosphatase carboxylase large subunit gene (rbcL). Figure 2A shows a diagram of the ribosomal RNA gene and spacer configuration and the position of the primers used for PCR amplification and DNA sequencing. Oligonucleotide primers plt08 and plt09 are highly conserved, universal sequences that are complementary to the rRNA loci of most plant species (Palumbi, 1996). These primers amplify a product of ~650 base pairs. The plt06 and plt07 oligonucleotide primers (Figure 2B) are complementary to the chloroplast rbcL gene and are also highly conserved (Palumbi, 1996). The plt06/plt07 primers amplify a PCR product of ~1500 base pairs.

Figure 3 shows the amplification results for the first 20 ginseng samples at both the ITS (A) and the rbcL (B) genes. Results show that the ITS primers were able to amplify all of the ginseng samples, although at different efficiencies. The larger chloroplast rbcL locus was amplified in all samples except sample 4. There is no general trend between the quality/quantity of genomic DNA isolated and the amount of PCR product generated. At the ITS locus, samples 1, 4, 9, and 12 were amplified at a lower relative rate than other samples, although the DNA preparations were similar to other well-amplified samples. As an example, ginseng sample 4 shows significant genomic DNA (Figure 1, lane 4) yet has a relatively poor quantity of PCR amplified product (Figure 3, lane 4). Other samples such as sample 2 have excellent PCR amplification yet almost no visible genomic DNA. Both of the samples labeled Red Korean (12 and 19) gave relatively poor ITS PCR amplification results compared to the other Korean samples. The rbcL PCR tends to correlate with the ITS PCR results, except that the Red Korean samples were well amplified. One notable exception is sample 20, which gave a reasonably
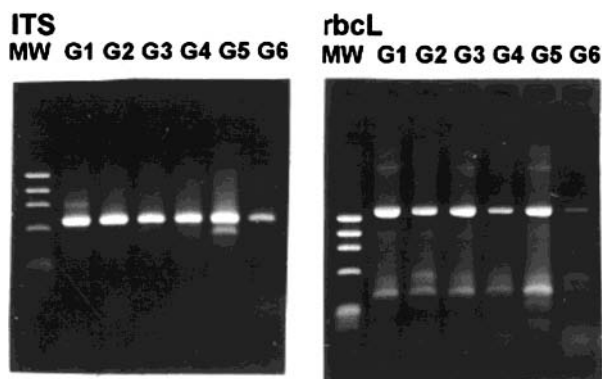
Figure 4. Agarose gel electrophoresis of PCR results (ITS and rbcL) from commercial ginseng samples G1–G6. MW refers to *Phi*X174/*Hae*III molecular weight markers.

good ITS signal but a very poor rbcL PCR amplification product.

PCR amplification products for the ginseng samples (G1–G6) that were purified by impact homogenization are shown in Figure 4. A strong PCR signal was observed for both the genomic ITS locus and the plastid rbcL gene. There is a dramatic improvement in signal intensity, including some secondary PCR products with these higher quality DNA preparations. The impact homogenization samples also improved the quality of the DNA sequencing results, and the secondary PCR products did not interfere in the nested primer extension sequencing reactions.

DNA Sequence Analysis and Species Identification. To identify and confirm the species of the different ginseng samples, we performed a direct DNA sequence analysis of the ITS PCR product. Sequence analysis of a portion of the chloroplast rbcL gene was also performed but was found to be less informative than the ITS locus (data not shown). An analysis of the two published ITS sequences for *P. quinquefolius* and *P. ginsneg* revealed a high degree of sequence identity. Of the 619 bases in the GenBank database entries, 615 were identical. The four nucleotide base differences were located at positions 118, 237, 416, and 425 (see Figure 2) of the published ITS region (Wen and Zimmer, 1996). DNA sequencing primers plt10 and plt11 were designed to give unambiguous sequence data at these nucleotide positions.

Table 1 shows the DNA sequencing results for all of the ginseng samples used in this study. We were able to accurately identify all of the samples based on the ITS DNA sequence analysis. Ginseng samples 1–10, all labeled American ginseng by their commercial suppliers, were identified as being American ginseng by the nucleotide sequence at the corresponding four identification positions. We correctly identified DNA samples that were isolated from fresh root, dried root, powder, and capsules. Korean-labeled samples 11–14, 17, and 18 were identified as being Korean ginseng by sequence analysis. Sample 15 (source E), labeled Chinese ginseng, was also identified as *P. ginseng*.

Two Korean ginseng samples were of particular interest. Samples 16 and 20 both gave excellent ITS PCR signals but gave no DNA sequence information when subjected to dideoxy primer extension sequencing with either the plt10 or plt11 primer. We then sequenced the ITS PCR product using the original PCR plt08 and plt09 end primers. This gave an unambiguous DNA electropherogram that was used in a GenBank

BLAST search. The results were highly significant for a similarity sequence match to the plant genus *Glycine* (soybean). To make sure that this sequence match was not an artifact, we repeated the PCR amplification and sequence analysis for both samples and confirmed that both PCR products were amplified from soybean DNA.

RAPD Analysis. To determine if ginseng species identification from commercial samples could be accomplished by genetic methods other than direct DNA sequencing, we performed a RAPD PCR analysis of our American and Korean ginseng samples (Shaw and But, 1995; Shoyama et al., 1997). An initial screen for potentially informative polymorphisms used 40 different 10-base oligonucleotides as random primers and 2 different DNA templates (American ginseng sample 6 and Korean ginseng sample 13) (data not shown). Four of the RAPD oligonucleotides (A2, Z8, Z10, and Z20) gave interesting PCR fingerprints and were subsequently used to screen the 20 commercial ginseng samples. Results were variable, with a few DNA templates giving high-quality RAPD fingerprints with the remaining samples giving poor-quality or no RAPD fingerprint (data not shown). Because many of the original 20 ginseng samples gave poor RAPD fingerprints but did give good nuclear ITS and chloroplast rbcL PCR amplification products, we concluded that the quality of the genomic DNA was a major issue with our RAPD analysis. These results initiated using the impact homogenization technique for DNA isolation (ginseng samples G1 to G6). As stated previously, this technique significantly improved DNA yield and PCR amplification quality. The G1–G6 ginseng samples were then subjected to RAPD analysis using the four 10-base oligonucleotide primers mentioned previously (Figure 5). High-quality fingerprints were generated for each sample tested. Primer Z20 gave a single band at ~550 base pairs for both the American and Korean ginseng samples. The other primers gave polymorphic DNA fingerprints that could be used to easily distinguish American from Korean ginseng. Primer A2 gave a band at ~400 bp that was specific for the American species. Interestingly, primer A2 amplified a ~600 bp fragment that was present in all Korean samples, but only in American sample G2. Primers Z8 and Z10 gave the most informative RAPD fingerprints. Each gave a PCR fragment that was present only in either the American or the Korean ginseng samples. The RAPD fingerprint for Z8 was especially dramatic in its ability to distinguish between the species. The RAPD fingerprint pattern for the two samples identified as soybean (samples 16 and 20) were not informative for sample identification (data not shown). Sample 20 gave no amplification products during the RAPD analysis, which is most likely due to degradation of the template DNA (Figure 1A).

HPLC Analysis of Commercial Ginseng Samples. A chromatographic analysis of the commercial ginseng samples was performed to determine if the previously identified ginseng biochemical markers, the ginsenosides, would correlate with our molecular genetic analysis (Breemen et al., 1995; Li et al., 1996; Wang et al., 1999). Twenty-two commercial samples (1–20, G3, and G6) were subjected to ethanol extraction and then analyzed by reverse-phase HPLC. Representative chromatograms of American and Korean ginseng samples are shown in Figure 6. Specific ginsenosides are distinguished by different retention times, and purified standards were used to calibrate our HPLC experiments (data not
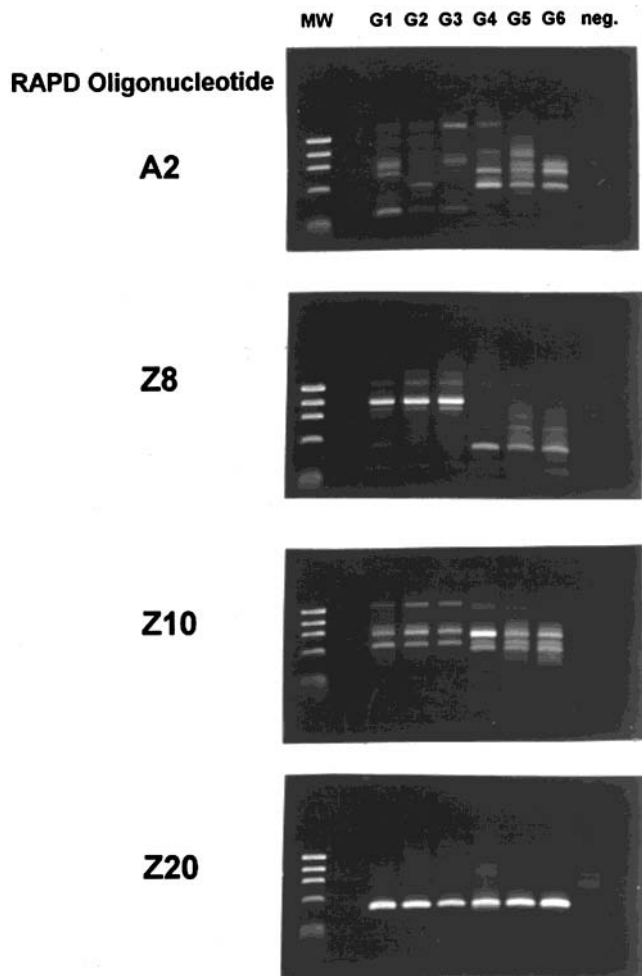
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Figure 5. Agarose gel electrophoresis of RAPD PCR results using four different 10-base oligonucleotide primers (A2, Z8, Z10, and Z20). Ginseng samples are G1–G6. Negative control (neg.) is a RAPD PCR reaction without template DNA. MW refers to *Phi*X174/*Hae*III molecular weight markers.

Figure 6. HPLC chromatograms of ethanol-extracted commercial ginseng samples: (A) American ginseng sample; (B) Korean ginseng sample. Ginsenosides Rf, Rb1, and Rc are labeled above their respective peaks. The *X*-axis shows retention time in minutes. The *Y*-axis shows relative UV absorbance at 203 nm (straight line) and percent acetonitrile (dashed line).

shown). Three ginsenosides were used in our analysis to distinguish American from Korean ginseng (Rf, Rb1, and Rc). As seen in Figure 6B, ginsenoside Rf is observed in only *P. ginseng* (Korean ginseng) with no peak observed at the ~45 min retention time point for the American sample (Figure 6A). Table 2 shows the results for the HPLC analysis of the commercial ginseng samples. All of the American-labeled samples showed no detectable ginsenoside Rf, whereas all genetically identified Korean samples showed a significant Rf peak. Samples 16 and 20, both genetically identified as soybean, showed no detectable ginsenosides of any type.

The ratio of ginsenoside Rb1 to ginsenoside Rc was also used to distinguish between the two species. Peak areas for the different ginseng samples were variable. These results are most likely due to sample extraction efficiency, injection volume variation, and overall sample ginsenoside concentration. However, percent peak areas are fairly constant and can be used for comparison. Table 2 lists the Rb1:Rc ratio of percent peak area for each sample. As seen in Table 2, the Rb1:Rc ratio is much higher in the American samples (4.4–12.0) than in the Korean ginseng samples (1.1–1.9).

DISCUSSION

We have described a molecular genetic procedure for the isolation and identification of American and Korean
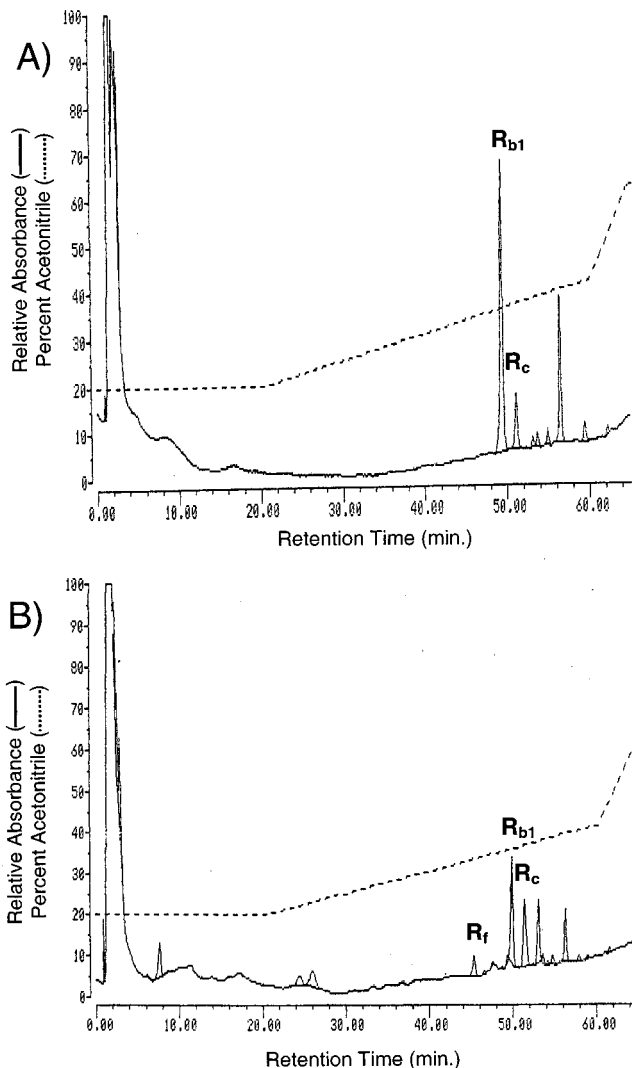
ginseng DNA from commercial suppliers. The types of samples analyzed include whole fresh root, whole dried root, powdered root, powdered root enclosed in a capsule, and tablet preparations. Building on previous molecular genetic research by other ginseng and plant researchers, we have combined and supplemented a number of protocols into a robust method for ginseng identification. Wen and Zimmer provided a detailed analysis of the phylogenetic relationships of all extant *Panax* species (Wen and Zimmer, 1996). Their work showed that the ribosomal ITS regions 1 and 2 were useful sequences in the identification and comparison of ginseng species. The medicinal ginseng types, Korean (*P. ginseng*) and American (*P. quinquefolius*), were shown to be genetically closely related, with only four nucleotide positions differing between the two species. On the basis of their work, we developed a nested PCR–DNA sequencing method that amplifies a single PCR fragment that can be sequenced in both directions using internal primers. Other workers have used the ribosomal 18S gene for sequence identification in ginseng samples. A nested primer approach using highly conserved genes such as rRNA 18S gene could potentially give significant back-

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Table 2. HPLC Data of Ginseng Samples: Ginsenosides Rf, Rb1, and Rc

sample	Rf min	peak area	Rf % area	Rb1 min	peak area	Rb1% area	Rc min	peak area	Rc % area	Rb1:Rc % area
1	none			50.85	30859	30.9	51.97	5883	5.9	5.2
2	none			50.90	309562	52.8	52.28	35100	6.0	8.8
3	none			50.51	207415	35.2	51.84	36043	6.1	5.8
4	none			50.25	190873	39.7	51.77	15714	3.3	12.0
5	none			50.38	117049	22.6	51.85	26374	5.1	4.4
6	none			50.20	180830	30.4	51.43	21304	3.6	8.4
7	none			49.47	181838	47.3	51.14	32776	8.5	5.6
8	none			50.12	118576	16.2	51.40	22880	3.1	5.2
9	none			51.40	188386	35.2	52.91	26666	5.0	7.0
10	none			50.47	282799	41.1	51.82	62465	9.1	4.5
G3	none			50.07	120607	42.0	51.61	14316	5.0	8.4
11	42.12	11408	3.2	48.36	76424	21.5	50.12	54730	15.4	1.4
12	43.44	10059	4.4	49.21	53690	23.6	50.81	50456	22.2	1.1
13	44.46	7686	2.9	49.84	46020	17.6	50.94	33053	12.7	1.4
14	45.34	9945	3.1	49.90	54779	17.0	51.40	41470	12.9	1.3
15	45.94	6370	3.5	49.86	45841	25.0	51.20	36562	19.4	1.3
16	none			none			none			
17	45.01	8499	4.4	49.86	60223	31.0	51.42	31200	16.0	1.9
18	43.06	4615	3.0	48.90	20377	13.1	50.31	10758	6.9	1.9
19	42.06	6727	5.9	49.56	49156	22.8	51.17	25773	12.0	1.9
20	none			none			none			
G6	45.39	5086	10.2	49.81	15665	31.4	51.19	8288	16.6	1.9

ground sequence noise because the sequencing primers could be complementary to a number of possible plant contaminants (Fushimi et al., 1996). Using the ribosomal ITS region as a target for nested sequencing primer binding gives great specificity in the sequencing reaction. As an example, ginseng samples 16 and 20 both gave good ITS PCR signals but no sequence information when primed with the plt10 or plt11 nested sequencing oligonucleotides. Only using the original PCR primers plt08 or plt09 were we successful in getting sequence information (data not shown). A number of other ginseng samples that gave excellent PCR signals gave inferior sequencing results when primed with plt08 or plt09 but gave excellent results when primed with either of the nested ITS primers.

Many plant species have thick cell walls and extracellular matrix material that can make DNA purification difficult (Demeke and Adams, 1992). We have incorporated glass bead impact homogenization in concert with a commercial plant-specific spin column chromatography method (Qiagen Plant DNeasy) to generate quality genomic and plastid DNA from different ginseng samples. RAPD PCR analysis was much improved when using template DNA purified by the impact homogenization step. One major benefit of glass bead cell disruption was the ability to maintain sterility from sample to sample. Glass bead impact homogenization used sterile, disposable 2 mL screw-capped tubes that obviated the need for cleaning and sterilization of other homogenization systems. (Kuske et al., 1998).

As shown from the genomic DNA gel electrophoresis results (Figure 1), the quality of DNA isolated from different ginseng samples was quite variable. It is well established that there is an inverse correlation between the amount of DNA degradation in a particular sample and the ability to amplify large PCR fragments (Lindahl, 1993). This observation has been confirmed in the analysis of the human mitochondrial DNA hypervariable regions in forensic and in ancient DNA studies (Krings et al., 1997; Poinar et al., 1998; Yang et al., 1997). The observation that RAPD PCR results are much improved upon impact homogenization is in good agreement with the concept that the improved quality of DNA allows for amplification of larger PCR fragments. This correlation is supported by our results with

ginseng samples G1 to G6. As seen in Figure 1B, the poorest quality DNA is from samples G4 and G6. In Figure 4, PCR amplification results show the weakest signals for both G4 and G6, especially for the relatively larger ~1500 base pair rbcL fragment.

RADP PCR has been used extensively to identify genetic samples from natural populations and in reconstructing genealogies (Grosberg et al., 1996). RAPD analysis has the benefit of being relatively inexpensive and easy to perform using just the PCR and gel electrophoresis. A number of criticisms concerning the interpretation of RAPD DNA fingerprints have been raised. The one most relevant to this study is that of contamination of the ginseng root material with other organisms. Even ginseng roots that are washed thoroughly might be expected to harbor bacterial, fungal, and protozoal contaminates that could give spurious amplification products when analyzed by RAPD PCR. In previous work, Shaw and But (1995) have shown that RAPD PCR using large primers (20- and 27-base oligonucleotides) work well in the amplification of ginseng samples isolated form dried or fresh roots. Normally, primers of that size are unlikely to generate many informative DNA fragments because the likelihood of a random perfect match is greater than 1 in a billion base pairs. In this study we screened our ginseng samples by RAPD analysis using 40 different, random sequence 10-base oligonucleotides. Although the initial 20 ginseng samples gave variable results, we were able to generate high-quality RAPD DNA fingerprints with DNA purified by impact homogenization. Primer Z20 (Figure 5) illustrates a fingerprint pattern that is homogeneous for all samples. At least for this analysis, there is no evidence of contamination. Primers Z8 and Z10 show distinct fingerprints for American and Korean ginseng samples. The Z8 fingerprint is especially dramatic in that both the presence and absence of species-specific PCR fragments identify each sample. The RAPD results for primer A2 are more complex. Both American and Korean samples can be differentiated in this analysis, but there are some bands that are shared between species. Although RAPD analysis can be used to identify ginseng samples when reasonably good quality template DNA is available, the technique is not useful for highly degraded DNA samples. As evidenced in our genomic

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DNA preparations from commercial ginseng samples, there is often significant degradation and/or low yield of template DNA. Molecular identification of these samples requires a directed PCR strategy such as the ITS or rbcL locus amplification methods employed in this study.

HPLC analytical results support all of the conclusions generated from our molecular genetic analysis of commercial ginseng species. Previous research has identified a number of ginseng specific compounds (the ginsenosides) that can be used to identify different ginseng species (Evans, 1996). One benefit of the molecular genetic approach to ginseng identification is best illustrated in the samples that were shown to contain soybean. Both capsule and tablet preparations (samples 16 and 20) gave a negative result with HPLC analysis, which could have a number of interpretations. DNA identification confirms the HPLC results with the additional information concerning the likely source of the "herbal" contaminant or adulterant in the sample.

A number of different types of ginseng are sold in the herbal marketplace. Ginseng species such as Korean ginseng are also sold as Chinese ginseng. Our results show that neither DNA testing nor HPLC analysis can distinguish between these two types. This result is anticipated because these ginseng types are the same species (*P. ginseng*). Our results have also shown that both white and red ginsengs are amendable to genetic identification. The ITS PCR signals from the two red ginseng samples in our study (samples 12 and 19, Figure 3) were similar to those of other "white" ginseng samples, although less intense than most samples. The rbcL PCR signal was also strong, indicating that steam treatment does not damage either nuclear or chloroplast DNA to a great extent. The observation that the rbcL PCR signal was more efficient than the ITS PCR signal for some samples may reflect the relative resistance of the circular chloroplast genome to degradation when compared to the linear nuclear DNA.

Other herbal samples should be identifiable by the same techniques illustrated in this work. Although HPLC analysis of herbal compounds requires a different protocol and different molecular standards for each herbal compound under study, direct DNA sequence analysis methods are the same for all herbal samples. Ribosomal ITS sequencing and RAPD PCR analysis are two methods that allow the identification of specific herbal species, even highly related ones. DNA identification of commercial herbal samples complements other analytical techniques and is a powerful tool that should play a major part in herbal medicinal quality control and validation.

ABBREVIATIONS USED

ITS, internal transcribed spacer; rbcL, ribulose 1,5-bisphosphate carboxylase large subunit; PCR, Polymerase Chain Reaction; RAPD, random amplified polymorphic DNA; HPLC, high-performance liquid chromatography; bp, base pairs; BLAST, basic local alignment search tool.

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